



## Cadmium Accumulation by Water Hyacinth and Salvinia Under Different Sulfur Concentrations

S. VESTENA,<sup>1</sup> J. CAMBRAIA,<sup>1\*</sup> M. A. OLIVA<sup>2</sup> & J. A. OLIVEIRA<sup>1</sup>

<sup>1</sup>Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, 365700-000, Brazil

<sup>2</sup>Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, 365700-000, Brazil

(Received May 18, 2007; Accepted August 1, 2007)

### ABSTRACT

Cadmium (Cd) accumulation by water hyacinth and salvinia under different sulfur (S) concentrations was studied. Plants were exposed during six days to 0 and 5  $\mu\text{M}$  of Cd in nutrient solution with 0, 400 and 800  $\mu\text{M}$  of S, and then S and Cd contents in tops and roots were determined. Sulfur content was always higher in S-treated plants, independent of the presence of Cd. Cadmium content in Cd-treated plants increased with the addition of S to the nutrient solution in both species. Water hyacinth roots accumulate an average of 1.4 time more Cd than those of salvinia. The increase in S concentration from 400 to 800  $\mu\text{M}$ , however, usually did not result in additional increase in S nor in Cd accumulation. The molar Cd/S ratio after Cd treatment reached average values of about 4.8 in roots and 2.6 in tops in comparison to their respective controls. Both species, therefore, were Cd accumulators and the accumulation was larger in the roots under presence of S. Since water hyacinth produced larger root mass and accumulated more Cd, it showed larger capacity to remove Cd from aquatic systems contaminated with this metal, especially if S is available.

*Key words:* aquatic plants, metal, phytoremediation, *Eichhornia crassipes*, *Salvinia auriculata*

### RESUMO

#### Acúmulo de cádmio em aguapé e salvinia sob diferentes concentrações de enxofre

Estudou-se o acúmulo de Cd em aguapé e salvinia sob diferentes concentrações de enxofre. As plantas foram expostas durante seis dias ao Cd nas concentrações de 0 e 5  $\mu\text{M}$  em solução nutritiva contendo S nas concentrações de 0, 400 e 800  $\mu\text{M}$  e, então, os teores de Cd e S foram determinados na parte aérea e raízes. Os teores de S foram sempre maiores nas plantas cultivadas com este elemento, independente da presença de Cd. Os teores de Cd em plantas tratadas com este metal aumentaram nas duas espécies com a adição de S. Raízes de aguapé acumularam, em média, 1,4 vez mais Cd do que as de salvinia. O aumento na concentração de S de 400 para 800  $\mu\text{M}$ , contudo, usualmente não resultou em aumento adicional nos acúmulos de S ou Cd. A relação molar Cd/S, após tratamento com Cd, atingiu valores médios de 4,8 nas raízes e 2,6 na parte aérea comparativamente aos seus controles. As duas espécies, portanto, foram acumuladoras de Cd, e o acúmulo foi maior na presença de S. Como o aguapé produziu maior massa radicular e acumulou mais Cd, mostrou maior capacidade de remover Cd de sistemas aquáticos contaminados com este metal, especialmente na presença de S.

*Palavras-chave:* plantas aquáticas, metal, fitorremediação, *Eichhornia crassipes* e *Salvinia auriculata*.

\*Corresponding author: José Cambraia, e-mail: cambraia@ufv.br.

## INTRODUCTION

Water is with no doubt the most critical substance for health, prosperity, and the sustenance of human endeavors. Although Earth seems to have an almost inexhaustible amount of water, only about 0.01% of all water is potentially available for human uses (Zeman *et al.*, 2006). Unfortunately, water is being contaminated worldwide by direct and/or indirect continuous input of pollutants into aquatic ecosystems with long-term implications on ecosystem functioning (Smolders *et al.*, 2004).

The increase in the environmental levels of metals has been of growing concern over the past fifty years. They are natural components of soil and rocks, usually occur at low concentrations and do not pose health risks to human and wildlife. However, due to anthropogenic activities, including mining, industrial processes, utilization in agriculture as herbicide or fungicide and others, the concentration of metals, such as cadmium (Cd), is becoming increasingly high (Davis, 1984). Cadmium is easily taken up by plant roots and translocated to above-ground tissues (Yang *et al.*, 1998), and then poses a potential threat to human health as it enters the food chain (Obata & Umebayashi, 1993).

Plant species show a wide variability in their capacity to uptake, accumulate and tolerate metals, including Cd (Hart *et al.*, 1998). Cadmium accumulated in plants can interfere with several physiological processes, resulting in declined productivity (Obata & Umebayashi, 1993). The processes of Cd uptake and accumulation are dependent on their concentration and exposure time, as well as on other factors such as redox potential, pH and concentration of other elements (Sanità di Toppi & Gabbrielli, 1999). Sulfur (S) nutrition seems to be one of the most important factors involved in Cd tolerance in plants. This element once absorbed, mainly as sulfate, is quickly reduced and assimilated into various sulfur compounds, including glutathione and phytochelatin, which are probably involved in plant tolerance to metals (Cobbet, 2000; Hall, 2002). In fact, it has been demonstrated that in tolerant plants exposed to Cd there was an increase in: a) mRNA encoding for the high-affinity sulfate transporter necessary for sustaining the higher sulfur demand (Nocito *et al.*, 2002); b) the activities of enzymes of the sulfur assimilation pathway such as ATP sulfurylase and APS reductase (Lee & Leustek, 1999); and also, c) phytochelatin biosynthesis (Benavides *et al.*, 2005).

The aquatic species *Eichhornia crassipes* (Mart.) Solms (water hyacinth) and *Salvinia auriculata* Aubl. (salvinia) are able to uptake and accumulate considerable amounts of the metal from the contaminated water without significant reduction in growth. Therefore they have been suggested as potential plants for phytoremediation or biological indication of polluted waters with Cd (Oliveira, 1998). Although many aspects of the mineral nutrition of these plants are known, little is known about the effects of sulfur nutrition on Cd absorption and accumulation and the potential use of these aquatic species

in bioremediation. Therefore, the objective of this work was to evaluate the influence of sulfur nutrition on Cd uptake and accumulation in two tropical aquatic species.

## MATERIAL AND METHODS

Aquatic plants of *Eichhornia crassipes* (Mart.) Solms (water hyacinth) and *Salvinia auriculata* Aubl. (salvinia), collected in ponds of the Universidade Federal de Viçosa (Viçosa, MG, Brazil) free of discharge of laboratorial or household wastewater, were used in this experiment. Plants were selected for uniformity in size and shape. Before any treatment, their surface was sterilized with 1% (v/v) sodium hypochlorite for 1 min, and immediately washed in running tap water and then rinsed and kept in deionized water for 24 h. Then, plants were transferred to a growth chamber at  $25 \pm 1^\circ\text{C}$  on a 16 h light/8 h dark cycle with a photon flux density of  $230 \mu\text{moles m}^{-2} \text{s}^{-1}$  for three days.

After this adaptation period, plants were transferred to polyethylene pots with 2.5 L of Hoagland's nutrient solution (Hoagland & Arnon, 1950), pH 7.0, with 1/5 of the original ionic strength, and exposed to three different sulfur (S) concentrations: 0, 400 and 800  $\mu\text{M}$  and two cadmium (Cd) concentration: 0 and 5  $\mu\text{M}$ . Sulfur solutions were prepared with  $\text{Na}_2\text{SO}_4$  and Cd solutions with  $\text{Cd}(\text{NO}_3)_2$ . P.A. grade salts in chloride form were used in nutrient solution preparation to avoid introduction of unwanted S. After 6 days of Cd exposure, plants were harvested, washed in tap running and deionized water, and immediately processed for the determination of S and Cd contents and dry weight. The roots were additionally washed with 0.1 N HCl for 10 min and rinsed in deionized water.

Plant tops and roots of the two species were oven-dried at  $80^\circ\text{C}$  and finely ground in an stainless steel electric grinder. Samples were digested in a nitric-perchloric acid mixture (2:1) (Allan, 1969). The extracts were analyzed for S by turbidimetry (Alvarez *et al.*, 2001) and Cd by atomic absorption spectrophotometry (Shimadzu AA-6701G). Results of S were expressed as % dry weight and Cd as  $\mu\text{g g}^{-1}$  dry weight.

The experimental design was a completely randomized ( $3 \times 2$  factorial with three replicates). The data were subjected to an analysis of variance (ANOVA) with a complementary test of Tukey (level of significance = 5%).

## RESULTS

In the absence of Cd, addition of S to the nutrient solution did not result in significant change in the yield of dry mass in none of the plant parts of the two species, except in the tops of water hyacinth in which an average increase of 22% was observed (Figure 1A and B). In the presence of Cd, the dry mass increased only in the tops of water hyacinth in the treatment with 400  $\mu\text{M}$  S. Top and root dry mass did not change with S treatment in Cd treated plants of salvinia. Cadmium treatment

reduced the dry mass yield only in the tops of water hyacinth in nutrient solution with 800  $\mu\text{M}$  S (Figure 1A). In the roots of water hyacinth and in the two parts of the salvinia plants no effect of Cd treatment was detected.

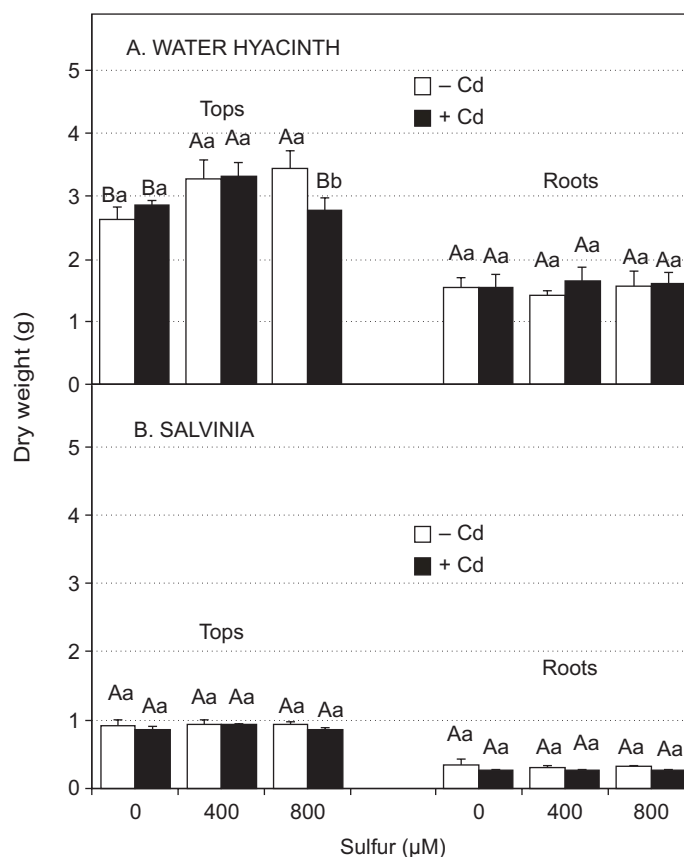
Water hyacinth, independent of S and/or Cd treatments, always showed larger shoot and root dry mass than salvinia (Figure 1).

In general, the contents of S in tops and roots of water hyacinth and salvinia increased with the addition of S to the nutrient solution, either in the absence or presence of Cd (Figures 2A and B). No significant differences were observed in S contents in tops of both species but in the roots S contents were higher in salvinia, independent of the presence of Cd.

In tops of plants not treated with Cd, S contents increased 120 and 150% in water hyacinth and salvinia, respectively, when S was added to the nutrient solution (Figures 2A and 2B). However, no additional effect was observed when S concentration was increased from 400 to 800  $\mu\text{M}$ . In Cd treated plants, increasing S concentration in the nutrient solution resulted in increased S content in both species, but especially in water hyacinth. In this specie an additional accumulation of 15% in S content was observed when S concentration was increased from 400 to 800  $\mu\text{M}$ , which was not observed for salvinia. In

roots of control plants treated only with S, contents of this element increased an average of 2.9 and 3.8 times in water hyacinth and salvinia, respectively (Figure 2). In water hyacinth no significant change was observed in S contents when the concentration was increased from 400 to 800  $\mu\text{M}$  (Figure 2A), while in salvinia a reduction of 27% was observed (Figure 2B). In Cd treated plants, S content increased an average of 85 and 102% with the addition of S to the nutrient solution in water hyacinth and salvinia, respectively. Increasing S concentration in the nutrient solution from 400 to 800  $\mu\text{M}$  did not change significantly S contents in roots of salvinia, although a reduction of 25% was observed in water hyacinth. In general, treatment with Cd resulted in increased S content in water hyacinth, but in a strong reduction in roots of salvinia in comparison to their respective controls.

In plants not treated with Cd, the contents of this metal were always low and did not change with the increase in the S concentration in the nutrient solution, independent of the species and of the analyzed plant part (Figure 3). Roots and tops of both plants showed much higher levels of Cd after exposure to this metal, which was independent of the presence of S in the nutrient solution (Figures 3A and 3B). Cd contents increased an average of 3.3 and 6.6 times and 3.1 and 4.8 times in tops and roots of water hyacinth and salvinia, respectively.



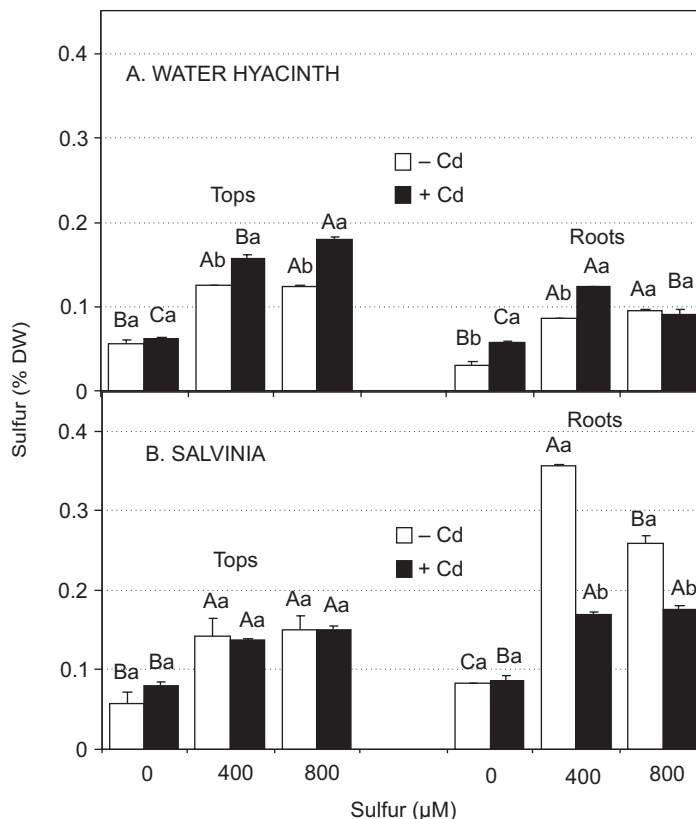
**Figure 1** – Average dry weight ( $\pm$ S.E.;  $n = 3$ ) of water hyacinth (A) and salvinia (B) plants after exposure to 0 ( $\square$ ) and 5  $\mu\text{M}$  ( $\blacksquare$ ) of cadmium for 6 days in nutrient solution with different sulfur concentrations. Means followed by same capital letter for S concentration within each Cd concentration and by same small letter for Cd concentration within each S concentration did not differ ( $p \leq 0.05$ ).

Addition of S to the nutrient solution resulted in an average increase of 14% and 25% in Cd content of water hyacinth and salvinia tops, respectively. In roots, the Cd content increased an average of 96% and 34% in water hyacinth and salvinia, respectively. No additional increase in Cd content, however, was observed increasing the S concentration of the nutrient solution from 400 to 800  $\mu\text{M}$  in water hyacinth, while a 15% reduction was observed in salvinia roots. The contents of Cd were similar in tops of both species (Figure 3), but 24% higher in roots of water hyacinth exposed to Cd. Cadmium content in roots was 2.8 and 1.9 times higher than in tops of water hyacinth and salvinia, respectively.

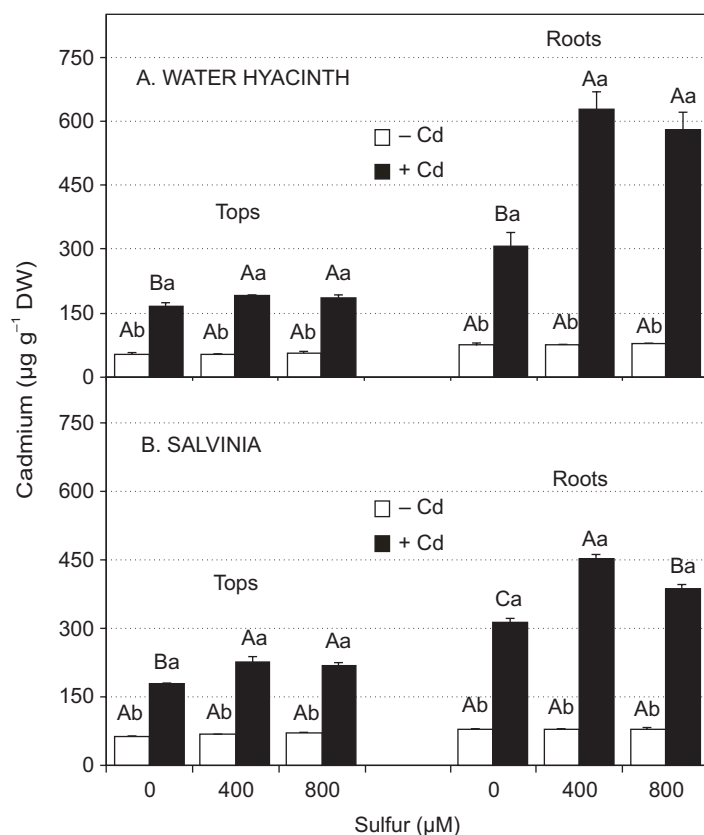
The average molar Cd/S ratios in Cd treated plants were about 2.7 and 2.6 times higher in tops and 4.0 and 5.6 times higher in roots than in control plants of water hyacinth and salvinia, respectively. In general, the addition of S to the nutrient solution in Cd treated plants resulted in a reduction of the molar Cd/S ratio, except in roots of water hyacinth where an increase of 18% was observed at the highest S concentration.

### DISCUSSION

Cadmium (Cd) is a non essential element that negatively affects the growth and the development of plants (Hall, 2002; Benavides et al., 2005). One of the main toxic effects of Cd in plants is growth inhibition in size and in dry weight of tops and roots (Benavides et al., 2005). In the present experiment, however, Cd reduced the dry weight only in tops of water hyacinth plants treated with sulfur (S) in the concentration of 800  $\mu\text{M}$ . In spite of that, symptoms of Cd toxicity were observed in both plant parts of the two species, especially in salvinia. These two aquatic species when exposed to increasing Cd concentration from 0 to 20  $\mu\text{M}$ , for 10 days, however, showed significant reduction in the rate of relative growth, as shown by Oliveira et al. (2001). Since plant responses to Cd exposure can largely vary among species, age of the plant, length of exposure, metal concentration and/or cultivation conditions (Chardonens et al., 1998; Soares et al., 2001; Ramos et al., 2002), it is possible that the applied Cd stress has not been severe enough to affect the dry weight of those plants in the present study.



**Figure 2** – Average sulfur contents ( $\pm$ S.E.; n = 3) in water hyacinth (A) and salvinia (B) plants after exposure to 0 ( $\square$ ) and 5  $\mu\text{M}$  ( $\blacksquare$ ) of cadmium for 6 days in nutrient solution with different sulfur concentrations. Means followed by same capital letter for S concentration within each Cd concentration and by same small letter for Cd concentration within each S concentration did not differ ( $p \leq 0.05$ ).



**Figure 3** – Average cadmium contents ( $\pm$ S.E.;  $n = 3$ ) in water hyacinth (A) and salvinia (B) plants after exposure to 0 ( $\square$ ) and 5  $\mu\text{M}$  ( $\blacksquare$ ) of cadmium for 6 days in nutrient solution with different sulfur concentrations. Means followed by same capital letter for S concentration within each Cd concentration and by same small letter for Cd concentration within each S concentration did not differ ( $p \leq 0.05$ ).

In plants grown in nutrient solution with no added S, the contents of this element varied from 0.03 to 0.08%. In spite of the fact that they were obtained from ponds apparently free of any laboratorial or domestic sewer, the plants were able to uptake some S, indicating a very efficient sulfate uptake system and capacity to store this element even in extremely diluted solutions. As expected sulfur content in S treated plants was always higher than in plants growing in the absence of this element. A two-fold increase in sulfate concentration in the nutrient solution, however, did not result in a significant increase in plant S content, suggesting that sulfate uptake system was already saturated. Sulfate uptake by plants is mediated by a  $3\text{H}^+/\text{SO}_4^{2-}$  symport, having  $K_m$  between 10 and 100  $\mu\text{M}$  (Leustek & Saito, 1999). In water hyacinth and salvinia the  $K_m$  of the sulfate uptake system was found to be 27 and 30  $\mu\text{M}$ , respectively (Oliveira *et al.*, 2007). Therefore, in the S added nutrient solution the sulfate concentration was higher than the root capacity to absorb this anion and, probably, for this reason increased S concentration in the nutrient solution did not result in a proportional increase in its content in the plants.

The tolerance of several plant species to metals such as Cd seems to be linked to S metabolism (Mendoza-Cózatl *et al.*, 2005). Under Cd stress, tolerant species increase sulfate uptake (Nocito *et al.*, 2002) and the activity of the enzymes that catalyze sulfate reduction (Harada *et al.*, 2002), and induce biosynthesis of low molecular weight peptides, called phytochelatins (Benavides *et al.*, 2005; Mendoza-Cózatl *et al.*, 2005). The increase in S content observed in water hyacinth was coherent with the induction of such mechanism of Cd tolerance, involving the use of reduced S. In salvinia, on the other hand, this does not seem to be happening. While no effect was observed in tops, there was a strong reduction in the S content in roots of Cd treated plants, suggesting a toxic Cd effect on the uptake and assimilation of S in this specie. The molar Cd/S ratio increase in the two parts of the treated plants of both species, suggesting that Cd accumulation may be related to other tolerance mechanisms, such as Cd immobilization to the cellular wall components and/or to extracellular carbohydrates (Sanitá di Toppi & Gabbrielli, 1999; Benavides *et al.*, 2005). The predominantly accumulation of Cd in the roots of both aquatic species reflected their ability to retain

more Cd than the shoot, which is not an exclusive property of water hyacinth and salvinia, but shared by almost all the plant species, including the terrestrial ones (Benavides *et al.*, 2005). In addition of having larger mass of roots than salvinia, water hyacinth was capable to support higher amounts of Cd in this tissue with no evident damage to its growth. For this reason, water hyacinth plants do have a higher potential as phytoremediator of aquatic systems contaminated with Cd than salvinia (Ding *et al.*, 1994; Oliveira, 1998).

## CONCLUSIONS

Water hyacinth and salvinia growth did not change by exposure to Cd under the applied experimental conditions. Both species, however, absorbed and accumulated large amounts of Cd, exhibiting potential to be used as phytoremediators. Water hyacinth, however, produced larger root mass and accumulated more Cd, showing higher capacity to remove Cd from aquatic systems contaminated with this metal than salvinia, especially if S is available.

*Acknowledgements* — The authors are thankful to FAPEMIG for financial support and CNPq for fellowships (2<sup>nd</sup> and 3<sup>rd</sup> authors).

## REFERENCES

- ALLAN, J. E., 1969, *The preparation of agricultural samples for analysis by atomic absorption spectrometry*. Varian Techtron Bulletin 12/69, 15p.
- ALVAREZ, V. H., DIAS, L. E., RIBEIRO, E. S., SOUZA, R. B. & FONSECA, C. A., 2001, *Métodos de análise de enxofre em solos e plantas*. Viçosa, UFV, 131p.
- BENAVIDES, M. P., GALLEGO, S. M. & TOMARO, M. L., 2005, Cadmium toxicity. *Braz. J. Plant Physiol.*, 17: 21-34.
- CHARDONNENS, A. N., BOOKUM, W. M. T., KUIJPER, L. D. J., VERKLEIJ, J. A. C. & ERNST, W. H. O., 1998, Distribution of cadmium in leaves of cadmium tolerant and sensitive ecotypes of *Silene vulgaris*. *Physiol. Plant.*, 104: 75-80.
- COBBET, C. S., 2000, Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.*, 123: 825-832.
- DAVIS, R. D., 1984, Cadmium – a complex environmental problem: cadmium in sludge used as fertilizer. *Experientia*, 40: 117-126.
- DING, X., JIANG, J., WANG, Y., WANG, W. & RU, B., 1994, Bioconcentration of cadmium in water hyacinth (*Eichhornia crassipes*) in relation to thiol group content. *Environ. Pollut.*, 84: 93-96.
- HALL, J. L., 2002, Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.*, 53: 1-11.
- HARADA, E., YAMAGUCHI, Y., KOIZUMI, N. & SANO-H., 2002, Cadmium stress induces production of thiol compounds and transcripts for enzymes involved in sulfur assimilation pathways in *Arabidopsis*. *J. Plant Physiol.*, 159: 445-448.
- HART, J. J., WELCH, R. M., NORVELL, W. A., SULLIVAN, L. A. & KOCHIAN, L. V., 1998, Characterization of cadmium binding, uptake and translocation in intact seedlings of bread and durum wheat cultivars. *Plant Physiol.*, 116: 1413-1420.
- HOAGLAND, D. R. & ARNON, D. I., 1950, *The water-culture method for growing plants without soil*. University of California, Agricultural Experiment Station, Circular n. 347, 39p.
- LEE, S. & LEUSTEK, T., 1999, The effect of cadmium on sulfate assimilation enzymes in *Brassica juncea*. *Plant Sci.*, 141: 201-207.
- LEUSTEK, T. & SAITO, K., 1999, Sulfate transport and assimilation in plants. *Plant Physiol.*, 120: 637-643.
- MENDOZA-CÓZATL, D., LOZA-TAVERA, H., HERNÁNDEZ-NAVARRO, A. & MORENO-SÁNCHEZ, R., 2005, Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *FEMS Microb. Rev.*, 29: 653-671.
- NOCITO, F. F., PIROVANO, L., COCUCCHI, M. & SACCHI, G. A., 2002, Cadmium-induced sulfate uptake in maize roots. *Plant Physiol.*, 129: 1872-1879.
- OBATA, H. & UMEBAYASHI, M., 1993, Production of SH compounds in higher plants of different tolerance to Cd. *Plant Soil*, 155/156: 533-536.
- OLIVEIRA, J. A., 1998, Efeito do cádmio sobre a absorção, a distribuição e a assimilação de enxofre em aguapé (*Eichhornia crassipes* (Mart.) Solms) e salvinia (*Salvinia auriculata* Aubl.). Tese de Doutorado, UFV, 93p.
- OLIVEIRA, J. A., CAMBRAIA, J., OLIVA, M. A. & JORDÃO, C. P., 2001, Absorção e acúmulo de cádmio e seus efeitos sobre o crescimento relativo de plantas de aguapé e salvinia. *Rev. Bras. Fis. Veg.*, 13: 329-341.
- OLIVEIRA, J. A., CAMBRAIA, J., SOUZA, M. V. & OLIVA, M. A., 2007, Uptake and metabolism of sulfate in water hyacinth and salvinia in the presence of Cd. *Aquat. Bot.*, in press.
- RAMOS, I., ESTEBAN, E., LUCENA, J. J. & GÁRATE, A., 2002, Cadmium uptake and subcellular distribution in plants of *Lactuca* sp. Cd-Mn interaction. *Plant Sci.*, 162: 761-767.
- SANITÁ di TOPPI, L. & GABBRIELLI, R., 1999, Response to cadmium in higher plants. *Environ. Exp. Bot.*, 41: 105-130.
- SMOLDERS, R., BERVOETS, L. & BLUST, R., 2004, *In situ* and laboratory bioassays to evaluate the impact of effluent discharges on receiving aquatic ecosystems. *Environ. Pollut.*, 132: 231-243.
- SOARES, C. R. F. S., ACCIOLY, A. M. A., MARQUES, T. C. L. L. S., SIQUEIRA, J. O. & MOREIRA, F. M. S., 2001, Acúmulo e distribuição de metais pesados nas raízes, caule e folhas de mudas de árvores em solo contaminado por rejeitos de indústria de zinco. *Rev. Bras. Fis. Veg.*, 13: 302-315.
- YANG, M. G., LIN, X. Y. & YANG, X. E., 1998, Impact of Cd on growth and nutrient accumulation of different plant species. *Chin. J. Appl. Ecol.*, 19: 89-94.
- ZEMAN, C., RICH, M. & ROSE, J., 2006, World water resources: Trends, challenges, and solutions. *Rev. Environ. Sci. Bio/Technol.*, 5: 333-346.